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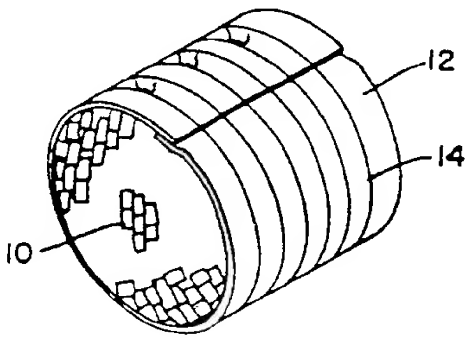
(54) Multi-tumour tissue slices, methods for their production and testing the tissues in such slices.

(57) A plurality of tissue rods have a relatively great length (e.g., 10 mm) and a relatively small cross-sectional area (e.g., 1mm²) in a transverse, preferably perpendicular, plane. The rods may constitute - (1) an array of well characterized neoplasms of varying histogenesis and degrees of differentiation and a spectrum of normal tissues, (2) groups of tissues with related types of tumors and/or normal tissues, - (3) tissue groups of defined classes and/or tissue groups as control samples and/or (4) representative tissue samples from defined patient populations. The rods are disposed in substantially parallel relationship in a casing having suitable dimensions (e.g., 2 cm x 3 cm). The casing may be formed from a s 'table material such as portions of small intestines of small animals such as rabbits and may be stretched, preserved and stored. The casing may be tightly wrapped around the rods and bound as by a fine thread. The ends of the rods and the casing may be trimmed to insure that the rod ends are exposed. When the rods are disposed in groups in the casing, septums in the casing may separate the different groups. The thread is removed and the combination is embedded as in paraffin. The paraffin block is then sectioned to produce one thousand - (1000) or more substantially identical slides. A single drop of a suitable material such as a hybridoma supernatant which may contain a monoclonal antibody may then be applied to individual ones of the slides to immuno react with the sectioned rods in the slides. The staining intensity of each rod section indicates the relative amount of the immuno reaction between the hybridoma supernatant and such rod section.

FIG. 4



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Multi-Tumor (Sausage) Tissue Slices and Methods of Producing such Slices and of Testing the Tissue in such Slices

This invention relates to multi-tumor tissue blocks and to methods of producing and using such blocks. The invention particularly relates to methods of producing a plurality of slides each containing a plurality of tissues with substantially identical characteristics to those of corresponding tissues in the other slides. In this way, all of the tissues in a slide can be tested with a single drop of a material such as a hybridoma supernatant.

In recent years, rapid progress has been made in applying immunohistochemical method to solving problems in histopathologic diagnosis. This progress has been largely stimulated by the development of hybridoma techniques for producing monoclonal antibodies. However, techniques for screening and applying such antibody have not kept pace with the techniques of producing such antibodies. Among the numerous new monoclonal antibodies, the identification of those clinically useful requires their specificity and sensitivity to be established by laborious immunohistological studies involving a large number of normal tissues and neoplasms. These studies have been generally performed on individual slides each of which contains a section from a single specimen.

Because of the use of slides with single tissue samples (or slices), the clinical studies now being performed have certain significant disadvantages. For example, the studies are slow and expensive because numerous slides have to be individually prepared and processed. The studies are also inherently limited in accuracy because each individual tissue section is not similar to the other slides. Limitations in accuracy also result because each slide cannot be processed in the same manner as the other slides.

A substantial effort has been made in recent years to provide methods and slides which will eliminate the disadvantages and limitations discussed above. In spite of such efforts and the considerable costs involved in such efforts, such disadvantages and limitations still persist.

In one embodiment of the invention, a plurality of tissue rods which have a relatively great length such as ten millimeters (10 mm) and a relatively small cross-sectional area such as one square millimeter (1 mm^2) in a transverse, preferably perpendicular, plane. The rods may constitute (1) an array of well characterized neoplasms of varying histogenesis and degrees of differentiation and a spectrum of normal tissues, (2) groups of tissues with related types of tumors and/or normal tissues, (3) tissue groups of defined classes and/or tissue groups as control samples and/or (4) representative tissue samples from defined patient populations.

The rods are disposed in substantially parallel relationship in a casing which may have dimensions of approximately two centimeters (2 cm) by three centimeters (3 cm). The casing may be formed from a suitable material such as portions of small intestines of small animals such as rabbits and may be stretched, preserved and stored. The casing may be tightly wrapped around the rods and bound as by a fine thread. The ends of the rods and the casing may be trimmed to insure that the rod ends are exposed. When the rods are disposed in groups in the casing, septums in the casing may separate the different groups.

The thread is removed and the combination is embedded as in paraffin. The paraffin block is then sectioned to produce as many as one thousand (1000) substantially identical slides. A single drop of a suitable material such as a hybridoma supernatant may then be applied to individual ones of the slides to react with the sectioned rods in the slides. The staining intensity of each rod section indicates the relative amount of reaction between the hybridoma supernatant and such rod section.

In the drawings:

Figure 1 is a schematic perspective view of a tissue rod which is included in one embodiment of the invention;

Figure 2 is a schematic perspective view illustrating a casing having a flat disposition and further illustrates the parallel disposition on the casing of a plurality of tissue rods corresponding to the tissue rod shown in Figure 1;

Figure 3 is a schematic perspective view of the arrangement shown in Figure 2 after the casing has been tightly wrapped and a thread has been wound around the tightly wrapped casing and the thread has been tied;

Figure 4 is a perspective view of the arrangement shown in Figure 3 after the ends of the casing have been trimmed to expose the ends of the tissue rods within the casing;

Figure 5 is a perspective view of the trimmed arrangement shown in Figure 4 after the thread has been removed and the arrangement has been embedded in a suitable material such as paraffin;

Figure 6 is a perspective view of slices of the embedded arrangement shown in Figure 5;

Figure 7 is an elevational view of one of the slices shown in Figure 6 and schematically illustrates the casing and the tissue rods within the casing;

Figure 8 is a schematic perspective view illustrating the application of a drop of a suitable material to be tested such as a hybridoma supernatant or an antiserum or other tissue reagent to the surface of a slice shown in Figures 6 and 7 to stain the exposed ends of the tissue rods in the slice;

Figure 9 is an elevational view schematically illustrating the staining of the exposed surfaces of individual ones of the tissue rods in the slice of Figures 6, 7 and 8 in accordance with the application of the hybridoma supernatant to the slice;

Figure 10 is an elevational view of a slice and schematically illustrates the disposition of a septum in the casing to separate the area within the casing into two (2) separate compartments; and

Figures 11 through 16 show slices actually obtained by applicant by practicing the invention as shown in Figures 1 through 10.

In one embodiment, a plurality of tissues 10 are provided. Each of the tissues may constitute rods having a suitable length such as approximately ten millimeters (10 mm) and a suitable cross-sectional area such as approximately one square millimeter (1 mm^2) in a plane transverse, preferably substantially perpendicular, to its length.

Each of the tissues may have been previously embedded in a material such as paraffin. The tissues are removed from the paraffin blocks in a conventional manner and deparaffinized in a material such as xylene. The tissues may then be rehydrated as in ethanol at a graded series of concentrations to a final concentration such as approximately fifty per cent (50%). The rehydrated tissue is then sliced as with a sharp razor blade into the sections which have a suitable thickness such as approximately one millimeter (1 mm). These sections are further divided as by a razor blade into the tissue rods 10 having approximately one square millimeter (1 mm^2) in cross section.

As many as one hundred (100) or more of the tissue rods 10 may be stacked in parallel on a casing 12. Preferably the tissue rods 10 are disposed in closely stacked relationship on the casing 12. The casing 12 may be prepared from a suitable material such as a portion of the small intestine of a small mammal such as a rabbit. Before the tissue rods are disposed in the casing 12, the material of the casing 12 is opened, stretched as over a cork board, preserved or fixed as in formalin and stored, until needed, as in a solution containing fifty per cent (50%) of ethanol. The casing 12 may have suitable dimensions such as approximately two centimeters (2 cm) by three centimeters (3 cm).

After the tissue rods 10 have been disposed in a closely stacked and substantially parallel relationship on the casing 12, the casing 12 is tightly wrapped around the tissue rods 10 and is maintained in this tightly confining relationship as by a fine thread 14. The ends of the resultant cylindrical package are subsequently trimmed as by a razor blade to expose the ends of all the rods at the exposed ends of the casing 12.

The fine thread 14 may thereafter be removed from the periphery of the casing 12. The cylindrical package containing the tissue rods 10 are subsequently embedded in a conventional manner into a block 16 made from a suitable material such as paraffin such that the ends of the tissue rods are substantially perpendicular to the face of the block 14 and are exposed at the face of the block. The paraffin block 16 may thereafter be sliced to form slices 18. The slicing is in a plane substantially perpendicular to the length of the rods 10. In this way, one thousand or more slices 20 can be produced from each paraffin block 16.

A suitable reagent such as a hybridoma supernatant 18 (Figure 8) or an antiserum or other reagents may be applied to the surface of the slices 20. Since the tissue rods 10 are closely spaced on the surface of each slice 20, only a drop of the reagent has to be applied to each slice. The reagents react with the tissue rods 10 and its presence may be detected with the application of further reagents to stain the exposed surfaces of the rods. The relative degree of such staining indicates the relative amount of reaction between the exposed surface of the tissue rods 10 and the reagent.

The tissue rods 10 may be disposed in different modes in the slice 20. In one mode, a broad array of tissue rods 10 constituting well characterized neoplasms of varying histogenesis and degree of differentiation may be provided in the slices 20. A wide spectrum of normal tissues may also be disposed in such slices. Typically, adenocarcinomas of various origins and squamous cell, undifferentiated and neuroendocrine carcinomas, lymphomas, melanomas, assorted sarcomas and samples of uncommon neoplasms may be disposed in the slices 20. These slices are particularly advantageous for for screening monoclonal antibodies in the early stages of hybridoma preparation. Tissue or tumor specificity, and any unexpected reactivity of these, can readily detected when a single drop of hybridoma supernatant is applied to these slices.

Figure 11 shows a slice 22 (magnified eight - (8) times) which contains a broad variety of samples of neoplasms. This slice has been immunostained in a conventional manner by the ABC method with a drop of a hybridoma supernatant obtained from spleen lymphocytes of a mouse

injected with human pancreatic carcinoma cells. After being stained by the hybridoma supernatant, the slice 22 was counterstained with hematoxylin. Only four (4) tissues (all gastrointestinal adenocarcinomas) show intense immunostaining . This indicates that a monoclonal antibody with some specificity is present in the supernatant.

Slices such as the slices 22 in Figure 11 have been used for immunohistochemical studies with many different antibodies. In such slices, tissues with several degrees of differentiation and with variable density of antigen expression have been provided within the slices. It has been possible to control the sensitivity of each such procedure and to monitor if there are any daily variations in the immunostaining method. Neoplasms with low antigen levels can be identified in each slice by their low staining intensity. If these neoplasms fail to stain the tissue rods in any particular slice, they provide an indication that the sensitivity of the procedure has decreased. Appropriate corrective measures can then be implemented.

Figure 12 is a close-up view, with a magnification of ten (10), of a portion of a slice 24 stained with a cocktail of monoclonal antibodies to keratins having a low molecular weight. The field shown in Figure 12 includes samples from fourteen (14) different epithelial neoplasms. The tissues in the slice 24 were stained in a conventional manner by the ABC method and were counterstained with hematoxylin. As will be seen, there is a marked variation in the intensity in which different tissues on the slice 24 are immunostained. Staining is particularly weak in two (2) undifferentiated carcinomas which are indicated by arrows. Tissues of such low antigen density provide a routine sensitivity control.

Slices such as the slices 22 and 24 containing miscellaneous types of tissues may be used for comparative studies of immunohistological methods. In particular, such slices may be used to resolve questions concerning the relative sensitivity of such immunohistological methods. Such slices may also be used as "check samples" in survey studies. For example, such slices may be used to provide inexpensive and reliable comparisons of the results of immunohistological studies among various laboratories.

Since there are a relatively large number of tissues in the slices such as the slices 22 and 24 and since many of these tissues contain similar morphologic features, it may not always be possible to identify the source of each individual tissue in such slices. Any such problem should be minimal, however, since the trained observer can recognize many classes of tumors with little difficulty. Furthermore, any inability to trace each tumor sample to its patient source should not be regarded as a serious impediment to the use of the method constituting this invention because such slices may generally be designed for screening and not for definitive studies.

The tissues 10 may also be disposed in segments such as in the slices 20. These segments may be obtained by wrapping groups of rods from related types of tumors or normal tissues in separate compartments within the casing 12. These compartments may be defined by septums 28 which may be constructed from the same material as the casing 12. This approach is advantageous because it provides for a grouping of related tissues and accordingly provides for an identification of a type of tissue or neoplasm from its position in the slice 20. This allows even technicians with no expertise in the tissue morphology to identify the different types of tissue in the slice and to interpret the results of the antibody screening on the tissues with minimal difficulty.

Figure 13 illustrates a slice 30 (with a magnification of eight (8)), containing seven (7) different compartments or segments. Individual ones of these compartments contain tissues with adenocarcinomas. The slice 30 was stained by the ABC with a heterologous antiserum to protein S100 and was counterstained with hematoxylin. Only the upper compartment shows intense immunostaining. This compartment contains tissues from six (6) different melanomas.

The segmented or compartmentalized slices discussed in the previous paragraph may be theme oriented. These slices are particularly useful for further characterization of antibodies which have shown apparent specificity in screening against tissues in such slices. For example, a slice 32 shown in Figure 14 has been used for comparing monoclonal antibodies specific to prostatic tissue. The slice 32 has been magnified fourteen (14) times.

In Figure 14, a septum 36 divides the tissues in the slice 32 into three (3) compartments. Such a septum is also schematically shown in Figure 10. The upper right compartment in Figure 14 contains nineteen (19) samples of prostatic tissues including well differentiated and poorly differentiated carcinomas. It also contains tissues of normal and hyperplastic prostate. The upper left compartment contains eight (8) samples of neuroendocrine carcinomas of various degrees of differentiation. These carcinomas have been chosen because of their histologic similarity to some prostatic carcinomas. The lower compartment contains twelve (12) samples of non-prostatic adenocarcinomas of various origins. The slice 32 of Figure 14 was obtained by immunostaining the tissues in the slice in a con-

ventional manner by the ABC method and then counterstaining the tissues with hematoxylin. A monoclonal antibody directed against prostatic-specific antigens was used in the immunostaining.

In addition to being used as controls for prostatic markers, the theme-oriented segmented slices may also be used as markers for endocrine differentiation. This is shown in a slice 40 in Figure 15, this slice being shown as being magnified fourteen (14) times. The slice 40 in Figure 15 was obtained from the same paraffin block as the slice 32 in Figure 14. As a result, the tissues in the slice 40 were substantially identical to the tissues in the slice 32 of Figure 14. The tissues in the slice 40 were obtained by the ABC method with a monoclonal antibody to neuron-specific enolase and was counterstained with hematoxylin. Staining was found to be intense only for several of the neuroendocrine carcinomas in the upper left compartment.

Slices may also be formed from tissues disposed in the slices in clinically defined segments. In these slices, representative tissue slices may be included from defined patient populations. For example, a slice 44, magnified seven (7) times in Figure 16, contained approximately ninety (90) tumor samples from two (2) groups of patients with stage I or stage II breast carcinoma. A single septum 46 separated the samples of the patients in one (1) group from the patients in the other group. One group included tissues of stage I and stage II patients who developed metastases or had recurrences in less than two (2) years after mastectomy. The other group was age-matched and stage-matched. The patients in this group were free of disease after a long period of follow-up. The slice was immunostained with a monoclonal antibody prepared against milk fat globule-derived membranes and was lightly counterstained with hematoxylin. Twice as many tumor samples were stained by the monoclonal antibody in the first compartment as in the second compartment. This suggests that the antigen being detected may have prognostic significance.

The tissue slices disclosed above have certain important advantages. One advantage is that errors resulting from procedural variations are effectively eliminated since all of the tissue samples in a slice are treated simultaneously by the same procedure. Quantitative digital readout of immunohistochemistry by microdensitometry may thus be enhanced from a reliability standpoint by the use of slices produced in accordance with this invention. Furthermore, slices out from a single block may be distributed to a number of histopathology laboratories as check samples. These slices may then be of great value as quality control tools in immunohistochemistry.

The small volume in each of the tumors sampled in the slices has not been a serious obstacle to the use of such tumors since these tumor samples have cross sectional areas of approximately one (1) square millimeter (1mm^2), this area is sufficiently large to fill a medium-power field of an average light microscope. Many endoscopic-biopsy specimens are not larger than this. Furthermore, sampling errors occur infrequently because the portions of tissue to be employed in the preparation of the slices are selected carefully.

Since only a small amount of hybridoma supernatant is available in the early phase of monoclonal antibody generation, immunohistologic screening is impractical unless a large number of tissues can be grouped within a small surface area. By this procedure, the amount of the hybridoma supernatant required to screen the hybridoma supernatant is minimized. This is accordingly one of the important advantages of this invention. For example, the slices of this invention can probably be used to detect tissue-specific antigens or tumor markers during the early stages of hybridoma preparation.

Although this invention has been disclosed and illustrated with reference to particular embodiments, the principles involved are susceptible for use in numerous other embodiments which will be apparent to persons skilled in the art. The invention is, therefore, to be limited only as indicated by the scope of the appended claims.

Claims

1. A method of preparing a multi-tumor tissue block, including the steps of:
providing a plurality of tissues having a relatively great length and relatively small cross-sectional area in a plane transverse to the length,
disposing the tissues in the plurality in spaced relationship on a casing so that the lengths of the tissues extend in substantially the same direction,
wrapping the tissues in the casing,
processing the wrapped casing in paraffin, and
sectioning the paraffin to obtain a plurality of samples.

2. A method as set forth in claim 1 wherein a fine thread is wrapped around the wrapped casing to retain the casing in a wrapped relationship.

3. A method as set forth in claim 1 wherein the casing is trimmed to expose the ends of the tissues before the wrapped casing is processed in paraffin.

4. A method as set forth in claim 1 wherein the casing is prepared from the portions of the small intestines of small animals such as rabbits.

5. A method as set forth in claim 4 wherein the casing is stretched, preserved and stored until it is needed.

6. A method as set forth in claim 4 wherein the rods have a length of approximately ten (10) millimeters and a cross-sectional area of approximately one square millimeter (1 mm^2).

7. A method of preparing a multi-tumor tissue block, including the steps of:
selecting tissues pre-embedded in paraffin,
removing the tissues from the paraffin blocks,
deparaffinizing the tissues in the paraffin block,
rehydrating the tissues,
forming the tissues into rods having a relatively great length and a relatively small area in a cross section transverse to the length,
disposing the rods in substantially parallel relationship on a casing,
wrapping the rods in the casing,
retaining the rods in the wrapped relationship in the casing,
embedding the wrapped rods as in paraffin, and forming thin sections of the wrapped rods in the pariffin.

8. A method as set forth in claim 7 wherein the ends of the rods are trimmed, before the wrapped rods are embedded in paraffin, to assure that the ends of the rods are exposed.

9. A method as set forth in claim 8 wherein the rods have a length of approximately ten millimeters (10 mm) and a cross-sectional area of approximately one square millimeter (1mm^2) and wherein
the rods are disposed in a substantially parallel relationship in the casing, and
the casing is tightly wrapped around the rods.

10. A method as set forth in claim 7 wherein the casing is obtained from portions of small intestines of small animals such as rabbits.

11. A method as set forth in claim 9 wherein the casing is obtained from portions of small intestines of small animals such as rabbits and wherein the casing material is stretched, preserved and stored before the tissue rods are disposed on the casing.

12. In a method of testing a plurality of tissues, the steps of,
providing a plurality of rods constituting tissue samples, each of the rods having a relatively great length and a relatively small cross-sectional area in a plane transverse to the length,
disposing the rods in a substantially parallel relationship in the casing,
tightly wrapping the rods in the casing,
embedding the rods in the tightly wrapped casing as in a paraffin block,
sectioning the block in planes substantially parallel to the cross sectional plane to form a plurality of sections.

13. in a method as set forth in claim 12,
disposing a drop of a hybridoma supernatant on the section of the block to react with the sections of the rods in the block.

14. In a method as set forth in claim 13 wherein
the rods are disposed in groups in each section of the block, the rods in each of such groups having different properties than the rods in the other groups.

15. In a method as set forth in claim 13 wherein
the rods are disposed in groups in each section of the block, the rods in each of such groups having properties different from, but related to, the properties of the rods in the other groups.

16. In a method as set forth in claim 12,
providing a septum in the casing, and
disposing in the casing a first group of the rods in the plurality on one side of the septum and disposing in the casing a second group of the rods in the plurality on the opposite side of the septum before tightly wrapping the rods in the casing, the rods in the first and second groups having properties different from, but related to, the properties of the rods in the second group.

17. In a method of testing a plurality of tissues, the steps of:
disposing sections of a plurality of tissues in a slice of a block, the tissue sections being disposed in groups each having properties different from, but related to, the properties of the tissue sections in the other groups, and
applying a hybridoma supernatant to the tissue sections in the slice of the block to react with the tissue sections.

18. In a method as set forth in claim 17,
evaluating the relative reactions of the hybridoma supernatant on the tissue sections in the different groups.

19. In a method as set forth in claim 18,
the tissue sections in the plurality being disposed in tightly spaced relationship in the section of the block,
one drop of the hybridoma supernatant being applied to the tissue sections in the section of the block.

20. In a method as set forth in claim 18,
the tissue sections in one of the groups constituting tissues of a particular type and the tissue sections in another one of the groups constituting a control

21. In a method of testing a plurality of tissues,
disposing a plurality of tissues in substantially parallel relationship in a paraffin block, each of the tissues constituting a rod having a relatively great

length and a small cross-sectional area in a plane transverse to the rod lengths,
sectioning the paraffin block in planes substantially parallel to the transverse plane to form a plurality of slices of the paraffin block, with the characteristics for different ones of the tissues in each slice being substantially identical to the characteristics of corresponding ones of the tissues in the other slices of the paraffin block, and
applying a hybridoma supernatant to the tissue sections in the different slices of the paraffin block to react with the tissue sections.

22. In a method as set forth in claim 21 wherein
the tissues constitute and array of well characterized neoplasms of varying histogenesis and degrees of differentiation.

23. A method as set forth in claim 21 wherein
the tissues are disposed in groups with related types of tumors.

24. A method as set forth in claim 21 wherein
the tissues are disposed in groups of defined classes including control groups.

25. A method as set forth in claim 21 wherein
the tissues constitute representative samples from defined patient populations.

26. In combination
a thin paraffin slice, and
a plurality of thin tissues disposed in spaced positions in the paraffin slice.

27. In a combination as set forth in claim 26,
a slice of a casing disposed in a closed loop in the thin paraffin slice,
the plurality of the thin sections of the tissue being disposed within the casing in the thin paraffin slice.

28. A combination as set forth in claim 26 wherein
a septum is disposed within the casing to divide the casing into at least a pair of compartments and wherein
tissues are disposed in each compartment with characteristics different from those of the tissues in the outer compartment.

FIG. 1

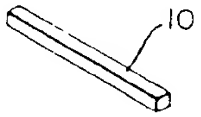
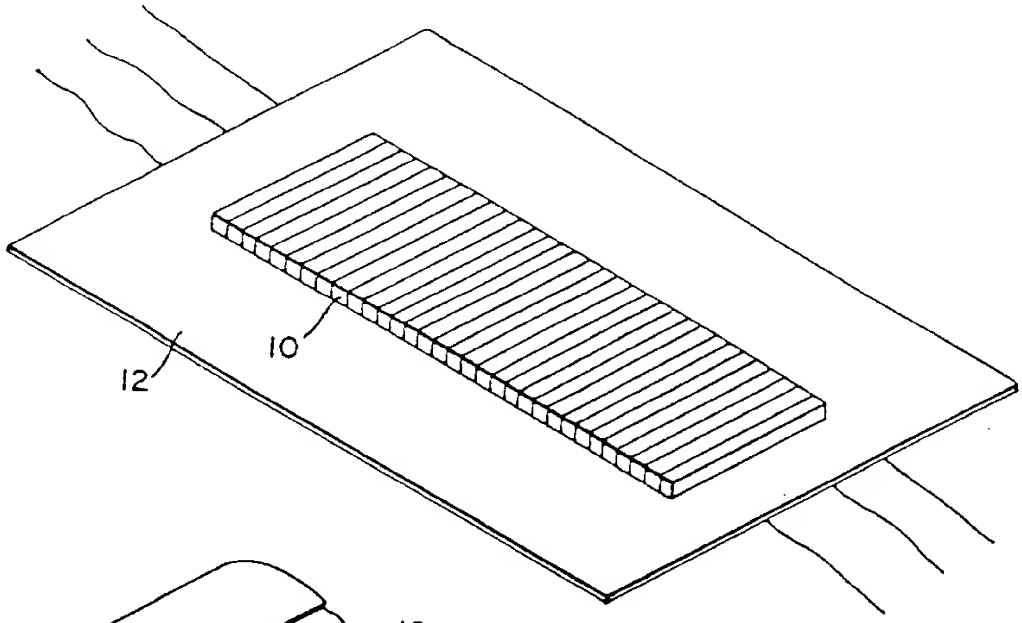


FIG. 2



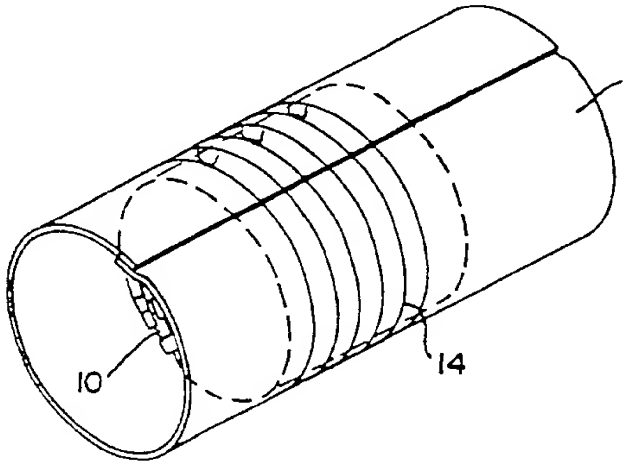
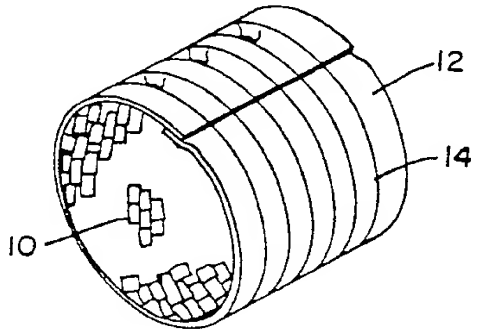


FIG. 3

FIG. 4



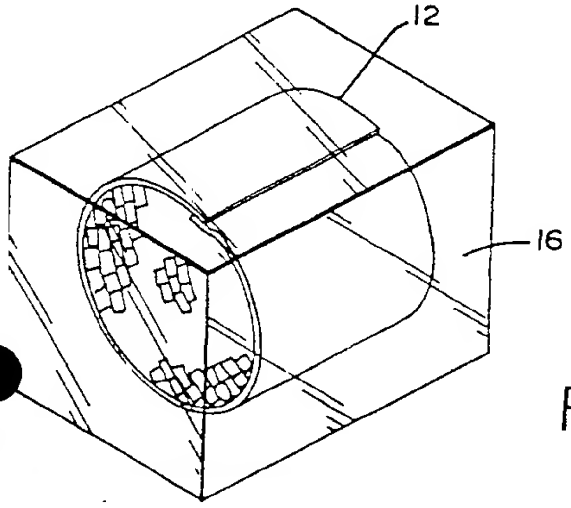


FIG. 5

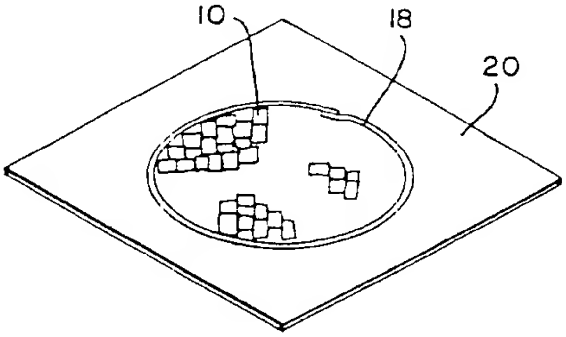


FIG. 6

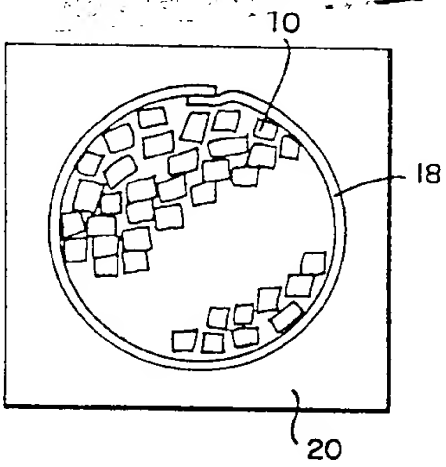


FIG. 7

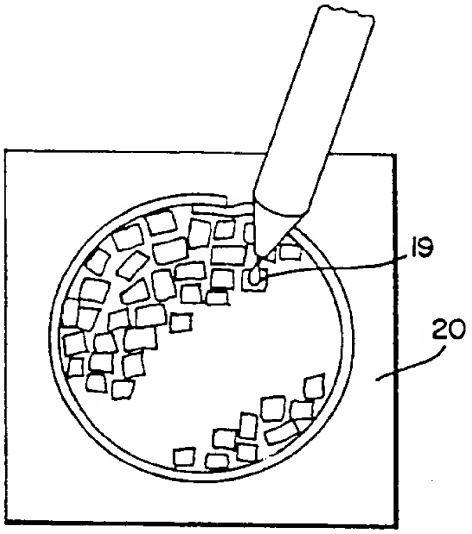


FIG. 8

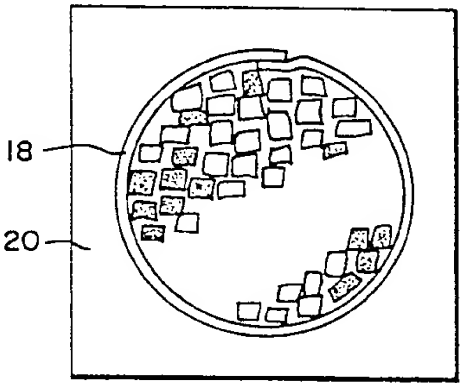


FIG. 9

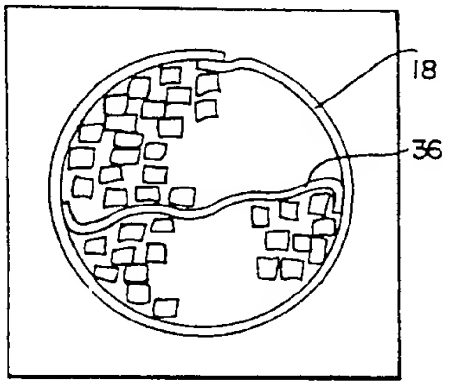


FIG. 10

FIGURE 11

FIGURE 12

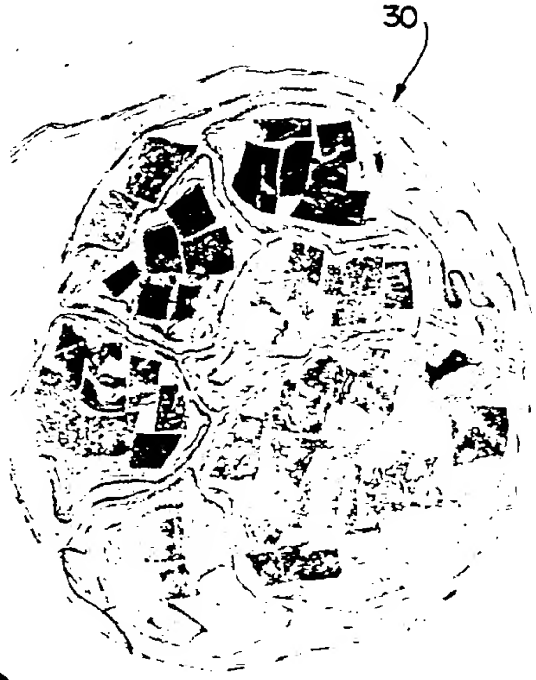


FIGURE 13

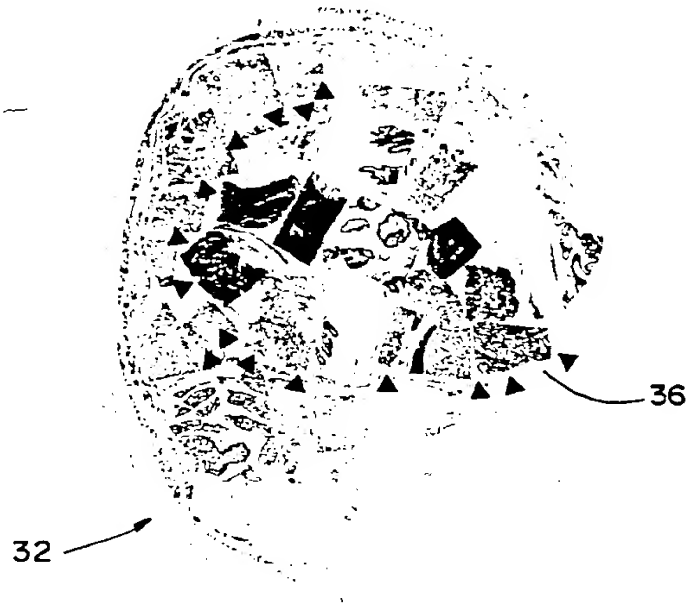


FIGURE 14

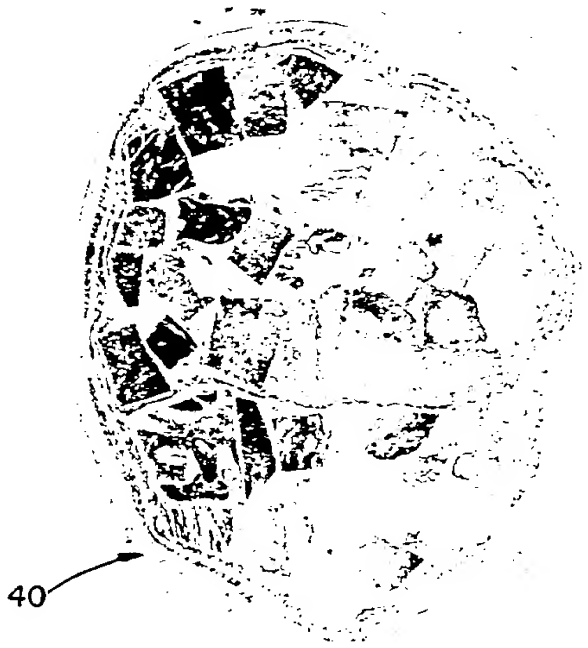


FIGURE 15

FIGURE 16